**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re Patent Application of

Tomi Järvinen et al.

Appln. No. 10/521,761 Group/Art Unit: 1623

Filed: January 21, 2005 Examiner: Goon, Scarlett

FOR: LIGNAN COMPLEXES

**DECLARATION OF JUKKA MÖNKKÖNEN**

Commissioner for Patents
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Sir:

I, Jukka Mönkkönen, declare the following:

1. I earned my Ph.D. in Pharmacology from the University of Kuopio, Finland, in 1991. Currently I am a Professor in Biopharmaceutics at the School of Pharmacy, University of Eastern Finland. I have almost 25 years research experience in molecular pharmacology, drug delivery and drug absorption. The aim of my research has been to understand the molecular mechanisms of drug absorption, permeation, metabolism, drug delivery and drug action. My curriculum vitae is attached.

2. I am not an inventor on the invention claimed in this patent application.

3. I have read and understand U.S. patent application S.N. 10/521,761, including the claims. I understand claims 13-20 have been rejected as obvious to a person

skilled in the art in view of US 2002/0061854 to Ahotupa et al. in view of Loftsson et al., J. Pharm. Sci., 85, 1017-1025 (1996). These claims have also been rejected as obvious over US 2001/0016590 to Ahotupa et al. in view of Loftsson et al

3.A. Both of the Ahotupa references disclose pharmaceutical preparations, food additives and food products which contain hydroxymatairesinol (HMR). However, neither of these references disclose or suggest the claimed inclusion complex of HMR or its ester and a cyclodextrin.

3.B. Loftsson et al. is a general review of pharmaceutical uses of cyclodextrin complexes, and it also fails to disclose the claimed inclusion complex of HMR or its ester and a cyclodextrin.

4. I believe the claimed inclusion complex of HMR or its ester and a cyclodextrin is non-obvious in view of the cited references for the scientific reasons set forth below

4.A. Cyclodextrins (CDs) are a group of structurally related oligosaccharides formed by enzymatic cyclization of starch by a class of amylases called glycosyltransferases (1,2). The important structural characteristics of the CD molecules are their cylindrical shape, somewhat hydrophobic central cavity and hydrophilic outer surface. Due to lack of free rotation of the bonds connecting the glucopyranose units, CDs are not perfectly cylindrical molecules but are, to some extent, cone shaped. All the primary hydroxyl groups are located on the narrow side while all the secondary hydroxyl groups are

located on the wider side of the cone. The most common natural CDs are α-CD, β-CD and γ-CD, consisting of 6, 7 or 8 α-1,4 linked glucopyranose units, respectively. These natural ("parent") CDs have rather limited solubility in water and, because of that, various CD derivatives have been made. The most common CD derivatives in pharmaceutical applications are nowadays hydroxypropyl-β-cyclodextrin (HP-β-CD) and sulfobutylether-β-cyclodextrin (SBE-β-CD).

4.B. The most important feature of CDs is their ability to form inclusion complexes with hydrophobic guest molecules (2). An inclusion complex is formed when the hydrophobic molecule, or part of the molecule, enters into the hydrophobic cavity of the CD. Thermodynamically, the formation of an inclusion complex is favorable because energy-rich water molecules inside the CD cavity are replaced by the hydrophobic guest. In addition, van der Waals interactions and hydrogen bonding between drug and CD, hydrophobic interactions, and the release of the strain energy of the CD molecule during the complexation have been proposed to contribute to inclusion complex formation.

**Unpredictability Relating to Complex Formation**

4.C. Loftsson et al. state the most common pharmaceutical application of cyclodextrin is to enhance drug solubility in aqueous solutions, yet concede that "prediction of compound solubilization by cyclodextrins continues to be highly empirical" (Page 1020, right col., lines15-16). In classical CD chemistry it is assumed that an inclusion complex between the drug and CD is always formed and drug/CD complexes are in ideal solution in which individual complexes are independent from each other. Recent studies have shown these assumptions are a significant oversimplification and that much more is involved in the complexation phenomena. It has been shown that CDs are able to form both inclusion and non-inclusion complexes in aqueous solution (3-5). In addition, CDs and their complexes have been shown to form water soluble aggregates in aqueous solutions and these aggregates are able to solubilize lipophilic drugs through micelle-like structures.

4.D. The simplest and most common inclusion complex contains one drug molecule and one CD molecule (2). However, the formation of higher order complexes (complexes which contain more than one drug and/or CD molecule) is common for molecules which are too large to fit completely in one CD cavity. For example, with large peptides it has been assumed that up to 40 CD molecules can interact at the same time with one peptide molecule.

4.E. These data clearly show that the CD complexation phenomena is complicated and difficult to predict. The basic requirement is that the drug molecule has to fit at least partly into the CD cavity. However, as discussed above, this is an oversimplification of the phenomena and usually hydrophobic interactions have been proposed to be the main driving force for CD complexation. Indeed, many drugs have very low complexation with CDs despite having structures which should fit well into the CD cavity. These compounds include, for example, benzodiazepines (6), acetylsalicylic acid (7), morphine (8), etc

4.F. Small structural changes in the guest-molecule have also been shown to have a significant effect on CD complexation, which makes predictions even more difficult. For example, Frömming and Szejtli (1) have demonstrated the role of substituents in complex formation with methylnitrophenols. Relative to 4-nitrophenol, methyl groups in position 2 and 6 have no significant influence on the stability of the α-CD complex, yet one methyl group in position 3 leads to about two orders of magnitude lower stability of the complex. In addition, 3,5-dimethyl-4-nitrophenol fails to give a complex at all.

**Unpredictability With Respect to Guest Molecule Stability**

4.G. Cyclodextrin interaction with labile compounds can retard their degradation, have no effect on their stability, or can accelerate drug degradation. See Loftsson et al., page 1021, right col., "Effect on Drug Stability". Thus, CD complexation can retard the degradation rate of many molecules (2). These results have been shown mainly in aqueous solutions, but also in solid inclusion complexes. Inclusion complex formation can be regarded as an "encapsulation" of the compound, or at least the labile part of it, at the molecular level. This encapsulation protects the molecule against attack by various reactive molecules, and thus, reduces hydrolysis, oxidation, steric re-arrangements, racemization, photodegradation or even enzymatic decomposition.

4.H. However, CD complexation can also *accelerate* degradation (1). This opposite effect on molecular stability has been explained by different structures of the inclusion complexes. For example, molecular degradation may increase if a hydrolytically labile ester linkage of the compound localizes in the vicinity of the hydroxyl group of the CD molecule. In contrast, if the same guest molecule penetrates deeper into the cyclodexrin, so that the ester linkage is in a apolar cavity beyond the reach of hydroxyl ions, deceleration of the hydrolysis is observed. The decrease in stability has been seen with small molecule drug compounds, such as β-lactam antibiotics (9), prostaglandins (10) and piroxicam (11).

4.I. The effect of CDs on guest molecule stability is very difficult to predict. Even in the simplest case where the compound has a hydrolytically labile ester bond, one should know the exact structure of the inclusion complex and where the labile bond is located in relation to the hydroxyl groups of the cyclodextrin molecule. Predicting a cyclodextrin's effect on a compound's stability is practically impossible if the compound has a more complicated degradation mechanism and multiple CD or guest molecules are involved in complexation.

**Conclusions**

5. In my opinion, one of ordinary skill would not have a reasonable expectation of success that cyclodextrins would form inclusion complexes with lignans and lignan esters from the Ahotupa references in view of Loftsson et al. The complexation of lipophilic compounds with CDs is a complicated reaction which is determined by various parameters. Various lipophilic drugs which should fit well into the CD cavity have been shown to have very low complexation constants with CD. In addition, small changes in a guest molecule's chemical structure may have a significant effect on CD complexation. If formed, the complex may contain more than one drug and/or CD molecule. Thus, all the scientific knowledge supports the fact that prediction of CD complexation is very difficult without laborious experiments in the laboratory.

6. In my opinion, the formation of "low solubility" cyclodextrin complexes with lignans and lignan esters with natural γ-CD is unexpected and surprising. These findings could not be predicted on the basis of common knowledge.

7. All statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true. These statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent resulting therefrom.

Signed this 6th of September, 2010.

Jukka MÖNKKÖNEN

Enclosures:
Curriculum vitae of Dr. Jukka MÖNKKÖNEN
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References

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